

The miRNA-Processing Enzyme Dicer Is Essential for the Morphogenesis and Maintenance of Hair Follicles

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Supplemental Experimental Procedures

miRNA-Array Analysis

Low-molecular-weight RNA was isolated with the mirVana RNA extraction kit (Ambion) from full-thickness dorsal skin of three K5-rtTA; tetO-Dkk1 newborn mice and three control littermates that had been doxycycline treated from E0.5 to induce expression of *Dkk1* in double-transgenic epidermis [S1]. Control and experimental samples were tagged for labeling with Cy3 and Cy5 dyes, respectively, by using the Ncode miRNA Labeling System (Invitrogen), mixed by equal mass, and cohybridized to miRNA arrays as described previously [S2]. Background-corrected median signals for pixels from each probe spot in both the Cy3 and Cy5 channels were used for analysis. Probes for each miRNA were spotted multiple times on the arrays. Control data were obtained from cohybridization of the same miRNA sample (self versus self). Data were analyzed with Spotfire DecisionSite version 8.2. Primary microarray data may be accessed in the GEO database (accession number GSE4723).

Generation of Mouse Lines and Genotyping

Dicer^{flox/+} ES cells were injected into mouse blastocysts and used to generate chimeric mice and a *Dicer*^{flox} mouse line via standard techniques [S3]. For generation of an epidermal-specific *Dicer* knockout, *Dicer*^{flox/flox} mice were crossed to K14-Cre line 52 transgenic mice [S4], and progeny heterozygous for both K14-Cre and *Dicer*^{flox} were crossed to *Dicer*^{flox} homozygotes. For PCR genotyping we used three primers: 23F (ATTGTTACCAGCGCTTAGAATTCC); 458F (TCGGAATAGGAACCTCGTTAAC), and 460R (GTACGTCTACAA TTGCTATG) (Figure 1D). Genomic DNA was extracted from epidermis and dermis that had been separated mechanically after incubation of skin overnight in 0.25% trypsin at 4°C. All experiments were performed with approved animal protocols according to the institutional guidelines established by the University of Pennsylvania IACUC committee.

Histology, Immunofluorescence, BrdU Incorporation, TUNEL Assays, In Situ Hybridization, and TEM

Tissue preparation and histology were as described previously [S4]. For immunofluorescence staining, sections were incubated with primary antibodies against keratin 1, keratin 10, keratin 6, involucrin, keratin 17, AE13, and GATA3 as described previously [S4, S5]. The following additional antibodies were used for immunofluorescence stainings: S100A6 (Neomarkers, Ab-9, 1:200 diluted), S100A4 (Neomarkers, Ab-8, 1:200 diluted), K15 (either from Vector Laboratories, VP-C411, 1:40 diluted or from Neomarkers, LKK15, 1:50 diluted), Ki67 (Vector Laboratories, VP-K452, 1:50 diluted), Sox9 Chemicon, AB5535, 1:100 diluted), phosphohistone H3 (Cell Signaling, 1:50 diluted), S100A3 [6], K14 (Covance, 1:500 diluted), and Cyclin D1 (Neomarkers, Sp4, 1:100 diluted). For alkaline phosphatase detection, sections were incubated in NBT and X-phosphate (Roche). BrdU assays and TUNEL staining were as previously described [S5]. Sections were mounted with Vectashield containing DAPI (Vector Laboratories). For quantitation of proliferation and apoptosis, numbers of proliferating or apoptotic cells were counted in at least six microscopic fields at magnifications of 20× (proliferation assays) or 10× (apoptosis assays). Nonradioactive in situ hybridizations with digoxigenin-labeled probes were performed as previously described [S1, S4]. The following PCR products containing a T7 promoter were used as templates for sense and antisense probe synthesis: *Shh* probe was as described previously [S4]; *Notch1* probe contained nucleotides 4728–5020 (accession number AF508809); and *Dicer* probe was generated by PCR with primers DicerF (GCAGGGCTTTAC ACACGCCCTCC) and DicerR (TTTGTAATACGACTCACTATAGGGCG TTCTGTGTCAGCCCCAAC). For TEM, tissue was fixed in 2% glutaraldehyde in 0.1% sodium cacodylate buffer, post-fixed in 1% osmium tetroxide, and embedded in Epoxy resin. Ultrathin sections were contrasted with double stains and examined with a FEI Tecnai-T12 transmission electron microscope.

Western Blotting, Northern Blotting, and RT-PCR

Dermis and epidermis (including hair-follicle epithelia) were separated mechanically after incubation of dorsal skin samples overnight

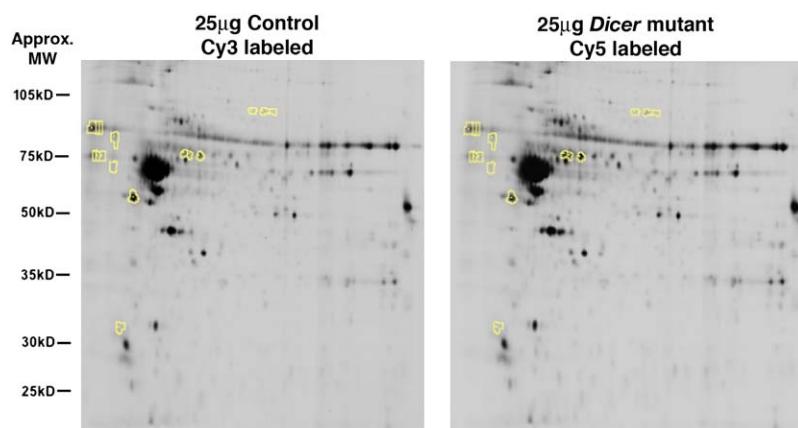


Figure S1. Protein Extracts from Control and Dicer Mutant Skin at P1 Analyzed by Two-Dimensional Gel Electrophoresis

Representative Coomassie-stained gels from triplicate experiments are shown. The highlighted spots show a statistically significant ($p < 0.05$) difference in expression of greater than 1.5-fold between mutant and control samples. None of the protein spots analyzed at this level of resolution showed statistically significant increases in expression of more than 1.8-fold in the *Dicer* mutant.

Table S1. miRNAs Listed According to the Average Probe Signal Strength Produced on Hybridization to Triplicate Samples of Control Newborn-Mouse-Skin Small RNAs

Name	Avg C	C2	C4	C5
hsa-miR-199a*	27.12789	26.81652	23.75632	30.81084
hsa-miR-199a*	25.07421	26.81775	21.44873	26.95615
hsa-mir-321	24.58406	24.07963	22.92543	26.74712
hsa-mir-321	24.31523	22.35236	24.33025	26.26308
hsa-miR-17-5p	24.13767	25.5428	19.4416	27.4286
hsa-mir-321	23.20337	23.91888	22.55854	23.13269
hsa-mir-321	22.24486	20.76575	23.05525	22.91358
hsa-miR-17-5p	20.5973	22.13584	16.60699	23.04907
hsa-mir-27	17.09518	16.43241	11.9481	22.90502
hsa-mir-27	16.40886	14.06993	10.56328	24.59337
hsa-mir-27	13.2827	13.14683	10.39449	16.30676
hsa-mir-27	12.09891	10.33244	8.953872	17.01041
hsa-mir-24-1,2	11.50961	10.47966	10.60788	13.44131
hsa-mir-203	10.23519	12.28894	6.81981	11.59682
hsa-mir-24-1,2	9.29591	7.252718	8.28821	12.3468
hsa-mir-203	9.266049	12.30042	5.256321	10.2414
hsa-miR-133b	8.360655	10.55265	9.842535	4.686778
hsa-mir-24-1,2	7.378791	7.878914	7.461253	6.796205
hsa-miR-127	7.301335	9.024274	7.566875	5.312857
hsa-miR-133b	7.040482	9.049289	8.098595	3.973561
miR-199-as	6.964665	8.797499	1.053735	11.04276
hsa-mir-91	6.924111	6.81311	9.252298	4.706925
hsa-miR-106ash	6.865526	9.244899	5.943627	5.408053
hsa-miR-27b	6.667448	6.334543	3.115122	10.55268
hsa-miR-27b	6.395177	6.76554	3.018231	9.401761
hsa-miR-127	6.152324	7.956009	6.072574	4.428388
hsa-let-7cL	5.979288	7.855539	3.255899	6.826426
mmu-mir-23b	5.92652	6.361609	7.28321	4.13474
let7A-biotin	5.843621	7.544287	1.370056	8.616518
let7A-biotin	5.692616	7.973233	0.989261	8.115353
hsa-mir-203	5.64883	6.202907	3.911113	6.83247
mmu-mir-23b	5.594234	6.656868	6.782885	3.342949
hsa-let-7cL	5.580923	6.81639	4.298216	5.628163
miR-199-as	5.493685	7.646397	1.660186	7.174472
mmu-mir-27b	5.38877	6.077012	5.377841	4.711458
hsa-mir-91	5.346514	6.429683	6.236689	3.37317
hsa-mir-24-1,2	5.313518	5.114138	5.701937	5.124479
hsa-let-7dL	5.289121	8.288586	0.557425	7.021352
let7A-biotin	5.261838	7.283064	3.770342	4.732109
hsa-mir-203	5.235264	6.51457	3.101686	6.089537
hsa-miR-106ash	5.144458	7.518862	4.81736	3.097152
hsa-miR-30a-5p	4.900148	4.18038	4.182714	6.337349
hsa-let-7a	4.895357	6.264419	2.250519	6.171134
miR-106b	4.795695	4.647054	4.653833	5.086199
hsa-let-7f	4.794237	4.693394	5.125942	4.563375
hsa-mir-26a	4.785355	5.606647	3.280923	5.468495
hsa-miR-143	4.768398	5.233062	3.593565	5.478568
hsa-miR-30a-5p	4.718875	4.581031	3.752505	5.823088
let7A-biotin	4.671746	5.906007	3.353952	4.755279
hsa-miR-143	4.623008	4.857837	3.197166	5.814022
miR-106b	4.574314	3.330279	3.123497	7.269164
hsa-mir-205	4.562558	4.256656	4.41181	5.019209
hsa-mir-91	4.561326	4.863988	5.503735	3.316254
mmu-mir-27b	4.510874	5.643555	4.281181	3.607887
mmu-mir-206	4.396382	3.943763	5.446096	3.799287
hsa-let-7dL	4.359288	7.06285	0.846211	5.168803
mmu-mir-27b	4.337414	5.365519	3.853481	3.793243
hsa-let-7a	4.321688	5.851466	2.689743	4.423855
hsa-mir-205	4.303713	5.839164	3.510931	3.561044
hsa-let-7f	4.249476	4.781151	4.076319	3.890957
hsa-let-7cL	4.245688	5.643965	2.104111	4.988988
mmu-mir-23b	4.044254	4.918529	4.451528	2.762706
mmu-mir-27b	3.989633	5.488133	3.157963	3.322802
hsa-let-7f	3.94875	5.107167	2.618949	4.120133
hsa-let-7a	3.939558	5.365929	1.992626	4.46012
mmu-mir-206	3.931056	3.93187	4.320906	3.540393
hsa-let-7f	3.87016	4.943544	2.584077	4.082861
miR-106a	3.840095	4.256656	0.177301	7.086327

Table S1. Continued

Name	Avg C	C2	C4	C5
miR-199-as	3.766117	5.104706	0.452656	5.740988
mmu-mir-23b	3.719169	5.425801	3.49938	2.232327
hsa-mir-30a	3.688138	4.253375	2.73523	4.075809
hsa-mir-219	3.684215	3.694433	3.420915	3.937296
hsa-mir-26a	3.632552	3.142052	2.537945	5.21766
hsa-mir-23b	3.620879	3.57961	2.556458	4.726569
mmu-mir-1d	3.617491	4.309966	2.366464	4.176042
hsa-let-7cL	3.588638	4.496144	3.128296	3.141476
hsa-mir-219	3.561742	3.442232	3.332393	3.910601
pred00211	3.489499	3.641122	4.071217	2.756158
hsa-let-7dL	3.426836	5.413908	0.300204	4.566397
pred00211	3.412927	3.129339	4.256577	2.852865
hsa-mir-205	3.398716	2.931269	4.213059	3.05182
hsa-let-7a	3.383215	4.816418	2.098067	3.235161
mir124-biotin	3.294992	4.37599	1.084628	4.424358
hsa-mir-91	3.236147	4.482611	3.14058	2.085251
hsa-mir-205	3.22651	3.698534	3.875597	2.105398
hsa-mir-26a	3.216877	4.314887	2.660679	2.675065
hsa-miR-422b	3.110141	3.735441	3.441229	2.153752
mmu-mir-1d	3.066425	3.409425	1.951277	3.838574
pred00143	3.039634	2.741401	2.895535	3.481966
hsa-miR-23a	2.984068	3.236371	2.398572	3.317261
hsa-mir-23b	2.975885	2.841461	2.107597	3.978598
mmu-mir-206	2.971257	3.209715	2.779666	2.924388
pred00209	2.891826	2.83367	4.874233	0.967577
mmu-mir-206	2.858667	3.359395	2.61256	2.604045
pred00209	2.814324	2.825468	4.719437	0.898068
mir124-biotin	2.802724	3.901524	0.792484	3.714164
miR-106a	2.776531	2.977609	0.313635	5.038349
hsa-mir-30a	2.758964	3.245803	2.117782	2.913307
pred00141	2.727483	3.037891	2.349617	2.794941
miR-127	2.724509	2.109464	4.465874	1.598189
hsa-miR-422b	2.6651507	3.379079	2.750091	1.82535
miR-106b	2.617864	2.008994	1.7975	4.047099
pred00141	2.6149	2.825058	2.420129	2.599512
miR-93	2.59598	2.43794	2.564628	2.785371
mmu-mir-1d	2.578981	3.665317	1.876069	2.195558
hsa-let-7dL	2.542433	4.239842	0.625256	2.762202
miR-127	2.520722	2.092651	3.90205	1.567464
hsa-mir-20	2.519772	2.14022	2.099315	3.31978
hsa-let-7bL	2.474391	3.498413	2.137691	1.78707
hsa-let-7bL	2.454501	3.269997	2.560797	1.53271
hsa-miR-23b	2.355727	2.925938	2.120464	2.020779
miR-199-as	2.354595	2.969817	1.023513	3.070456
hsa-miR-200c	2.342728	3.659986	2.173965	1.194234
hsa-miR-23a	2.328961	2.58516	1.799692	2.60203
miR-106b	2.311189	2.507244	2.222203	2.20412
hsa-miR-1	2.287939	1.324976	0.562446	4.976396
hsa-miR-1	2.272125	1.375006	0.495193	4.946175
pred00143	2.244682	2.026217	2.344546	2.363284
hsa-mir-26a	2.188086	2.280058	1.993447	2.290754
mmu-mir-1d	2.161684	3.093662	1.599786	1.791603
miR-93	2.150022	2.043441	2.010602	2.396024
hsa-mir-16	2.029549	2.124227	1.784981	2.17944
hsa-let-7bL	2.029334	2.941111	1.540642	1.606248
mmu-mir-133	2.006788	1.942561	1.463182	2.614623
hsa-mir-20	1.99602	2.33993	1.542228	2.105902
hsa-miR-200c	1.962025	3.113346	1.693335	1.079394
hsa-miR-23a	1.950905	2.683169	1.822192	1.347354
hsa-miR-181b	1.929644	2.662255	1.486178	1.640498
hsa-miR-23b	1.894151	2.301383	1.76072	1.620351
hsa-miR-181b	1.825697	2.582699	1.603452	1.290942
hsa-let-7bL	1.821726	2.560555	1.748165	1.156458
mmu-mir-133	1.815316	2.142271	1.239581	2.064096
hsa-mir-30a	1.811172	2.121356	1.40773	1.904428
hsa-mir-16	1.790118	2.013915	1.774871	1.581567
miR-106a	1.774366	2.346492	0.065816	2.910789
mmu-mir-143	1.772786	1.550522	1.247908	2.51993
miR-93	1.764503	1.580048	2.048785	1.664675
hsa-mir-16	1.761393	2.299742	1.062381	1.922057

Table S1. Continued

Name	Avg C	C2	C4	C5
hsa-mir-106	1.74056	1.562824	1.739318	1.919539
hsa-miR-23a	1.620219	2.210344	1.573438	1.076876
mmu-mir-143	1.583965	1.025616	1.240095	2.486183
miR-127	1.466866	1.367625	2.276439	0.756533
miR-127	1.462272	1.531248	1.907635	0.947933
hsa-mir-103-1,2	1.44664	1.585379	1.422801	1.33174
hsa-mir-16	1.445866	1.981108	1.051948	1.304541
hsa-miR-381	1.406113	1.483268	0.978221	1.756849
hsa-miR-324-3p	1.393705	1.750642	1.445269	0.985205
hsa-mir-20	1.387757	1.165864	1.3695	1.627906
miR-93	1.347792	1.401662	1.405171	1.236544
pred00049	1.329121	1.103122	0.825182	2.059059
hsa-miR-324-3p	1.309807	1.715785	1.370973	0.842663
pred00049	1.292007	0.967384	0.726679	2.181958
mmu-mir-133	1.286077	1.068265	1.358999	1.430966
hsa-mir-106	1.227656	1.187599	1.134415	1.360954
hsa-miR-381	1.227375	1.387309	0.83363	1.461187
miR-106a	1.221781	1.767455	0.227671	1.670215
hsa-mir-22	1.211718	0.863224	0.471103	2.300828
hsa-mir-20	1.172681	1.370906	1.09746	1.049677
hsa-mir-30d	1.160047	1.084258	1.12257	1.273313
mmu-mir-143	1.14523	1.279867	0.999364	1.156458
hsa-mir-30a	1.121221	1.351632	1.055032	0.956999
pred00127	1.118615	1.432828	0.912626	1.01039
hsa-miR-21	1.113514	0.752091	0.781738	1.806714
mmu-mir-133	1.106354	1.169965	1.096902	1.052195
CAND853_ZF	1.096075	1.34507	1.393635	0.549519
pred00127	1.091517	1.429547	0.875411	0.969591
hsa-mir-106	1.085259	0.99322	1.298508	0.964051
mmu-let-7iL	1.074122	1.523046	0.347433	1.351887
hsa-miR-200a	1.066603	1.218765	1.130826	0.850218
hsa-mir-26b	1.054334	1.520586	0.220014	1.422403
hsa-mir-103-1,2	1.031247	1.213434	1.145433	0.734875
hsa-mir-103-1,2	1.025196	1.162994	0.915804	0.99679
mmu-mir-30e	1.009112	0.991579	1.282749	0.753007
hsa-mir-22	1.008009	0.910793	0.469714	1.64352
mmu-let-7gL	1.005297	1.072366	0.454636	1.488889
mmu-let-7gL	0.983884	1.19416	0.249967	1.507526
mmu-let-7iL	0.977494	1.219175	0.631395	1.081913
mmu-mir-143	0.969255	0.79474	0.893607	1.219418
Near144a2	0.955527	0.241128	0.609207	2.016246
hsa-mir-30d	0.946542	0.816884	0.910103	1.112637
mmu-let-7gL	0.933662	0.917354	0.608808	1.274824
miR-143a	0.896183	0.804582	0.836809	1.047159
hsa-miR-200a	0.895268	1.078927	0.801991	0.804887
miR-30e	0.88582	0.758653	0.909068	0.989739
miR-199-s	0.883805	0.860763	1.371587	0.419065
hsa-miR-145sh	0.861553	1.019875	0.265281	1.299504
mmu-let-7gL	0.845008	0.771365	0.45257	1.311089
hsa-mir-214	0.844645	0.850921	1.057437	0.625575
hsa-mir-26b	0.834795	1.007162	0.18815	1.309074
hsa-mir-214	0.822028	0.819345	0.961728	0.68501
miR-30e	0.81897	0.751271	0.500324	1.205315
hsa-mir-18	0.795052	0.686068	0.920393	0.778695
hsa-mir-30d	0.793922	0.915304	0.822249	0.644212
Near144a2	0.790496	0.308382	0.474488	1.588619
CAND853_ZF	0.787327	1.09287	0.84652	0.422591
miR-94	0.763055	0.934578	0.528546	0.826041
mmu-mir-30e	0.76164	0.79802	0.906655	0.580244
hsa-mir-214	0.742802	0.783257	0.942473	0.502676
hsa-mir-103-1,2	0.728928	0.938679	0.69355	0.554556
hsa-mir-107	0.72627	0.754552	0.291473	1.132785
mmu-let-7iL	0.71851	0.866094	0.525851	0.763585
miR-143a	0.71659	0.715594	0.758232	0.675944
mmu-let-7iL	0.708225	1.015364	0.35983	0.749481
hsa-mir-106	0.69577	0.778336	0.72873	0.580244
hsa-mir-214	0.682842	0.737738	0.872584	0.438205
mmu-mir-30e	0.677625	0.674586	0.855614	0.502676
hsa-mir-18	0.669399	0.619224	0.715044	0.673929
hsa-mir-30d	0.663266	0.715594	0.703531	0.570674
miR-94	0.657632	0.782847	0.470789	0.71926

Table S1. Continued

Name	Avg C	C2	C4	C5
hsa-let-7eL	0.654103	0.771775	0.54884	0.641693
miR-199-s	0.648767	0.428126	1.189774	0.328402
miR-21	0.642278	0.39696	0.835294	0.69458
miR-94	0.633875	0.825906	0.712564	0.363156
hsa-miR-21	0.631792	0.631117	0.517801	0.746459
miR-199-s	0.62296	0.906282	0.623116	0.339483
hsa-mir-18	0.618402	0.590109	0.754865	0.510232
rno-miR-352	0.613703	0.786948	0.578181	0.475981
miR-30e	0.594567	0.600361	0.584963	0.598376
mmu-mir-126	0.581886	0.377686	0.442202	0.925771
hsa-mir-199a-1	0.562557	0.458472	0.372937	0.856262
hsa-miR-370	0.561204	0.544589	0.628791	0.510232
hsa-mir-31	0.560984	0.477336	0.587597	0.61802
hsa-miR-145sh	0.56022	0.686888	0.181331	0.812442
hsa-let-7eL	0.551269	0.817704	0.290613	0.54549
miR-143a	0.54702	0.572065	0.415718	0.653278
hsa-mir-22	0.54371	0.435508	0.327271	0.868351
hsa-mir-181a	0.538932	0.417054	0.644178	0.555563
miR-94	0.537985	0.6467	0.520487	0.446768
hsa-miR-370	0.537207	0.551151	0.547215	0.513254
hsa-mir-320	0.534018	0.588468	0.642372	0.371215
hsa-miR-134	0.530879	0.617994	0.503697	0.470944
hsa-mir-30c	0.530487	0.630297	0.469568	0.491595
miR-30e	0.527976	0.617174	0.329593	0.63716
rno-miR-352	0.526911	0.654902	0.500722	0.425109
hsa-mir-18	0.523071	0.572065	0.512604	0.484544
hsa-mir-107	0.521007	0.577806	0.37878	0.606435
rno-miR-351	0.520672	0.488408	0.656557	0.41705
hsa-let-7eL	0.519734	0.561813	0.527955	0.469433
hsa-miR-183	0.514545	0.791459	0.540628	0.211547
mmu-miR-195	0.505368	0.49784	0.360454	0.657811
hsa-mir-199a-1	0.498181	0.442889	0.376717	0.674936
hsa-mir-26b	0.496936	0.929657	0.090709	0.470441
mmu-mir-126	0.495863	0.441249	0.300888	0.745452
miR-298	0.495379	0.493739	0.598013	0.394384
hsa-mir-22	0.483195	0.451501	0.341784	0.6563
miR-21	0.481363	0.368254	0.597839	0.477996
hsa-mir-181a	0.480342	0.39614	0.655034	0.389851
hsa-mir-210	0.474643	0.587238	0.369273	0.467419
hsa-miR-34a	0.465912	0.673765	0.30692	0.41705
hsa-let-7eL	0.460574	0.59544	0.308791	0.477492
pred00153	0.455803	0.567964	0.446362	0.353082
hsa-mir-320	0.448738	0.561403	0.600968	0.183845
miR-130	0.446089	0.280086	0.647679	0.410502
hsa-miR-134	0.444598	0.5979	0.394899	0.340994
mmu-miR-30e	0.439459	0.493329	0.481535	0.343512
mmu-mir-200b	0.439122	0.485948	0.333779	0.49764
rno-miR-351	0.433425	0.39983	0.495483	0.404962
mmu-mir-195	0.432996	0.376046	0.259591	0.663352
hsa-mir-199a-1	0.428947	0.4474	0.418865	0.420576
pred00260	0.424657	0.190688	1.006722	0.07656
hsa-mir-107	0.423295	0.49538	0.255207	0.519298
hsa-miR-34a	0.420953	0.672535	0.245804	0.34452
miR-21	0.418246	0.423615	0.284627	0.546497
hsa-mir-31	0.417964	0.316994	0.414076	0.522824
hsa-miR-183	0.417884	0.667204	0.380441	0.206007
hsa-mir-320	0.41504	0.395319	0.493696	0.356104
pred00153	0.413851	0.485538	0.360623	0.395392
hsa-mir-181a	0.410042	0.3498	0.404345	0.475981
miR-298	0.403609	0.394909	0.518241	0.297677
miR-199-s	0.402178	0.480617	0.4227	0.303218
pred00260	0.399971	0.20135	0.911426	0.087137
hsa-mir-31	0.399762	0.391629	0.403702	0.403954
miR-298	0.399002	0.422385	0.393837	0.380785
rno-miR-351	0.394484	0.392449	0.542183	0.24882
miR-297	0.388589	0.517524	0.052385	0.595858
hsa-mir-210	0.387705	0.456832	0.422708	0.283574
hsa-mir-210	0.382704	0.504401	0.324374	0.319336
hsa-mir-199a-1	0.37956	0.392039	0.377944	0.368697
miR-300	0.373492	0.425666	0.310501	0.384311
mmu-mir-134	0.362168	0.532287	0.212216	0.342001

Table S1. Continued

Name	Avg C	C2	C4	C5
miR-130	0.355449	0.234567	0.564827	0.266952
hsa-mir-30c	0.354213	0.417874	0.296216	0.348549
hsa-mir-181a	0.352314	0.34693	0.399241	0.310773
Near144a1	0.344519	0.111132	0.211223	0.711201
miR-298	0.342957	0.419514	0.295562	0.313795
mmu-mir-30b	0.341899	0.301821	0.468005	0.255871
mmu-mir-200b	0.341501	0.428126	0.363172	0.233206
mmu-mir-195	0.339541	0.407212	0.259841	0.351571
hsa-miR-342	0.338861	0.343239	0.541378	0.131965
miR-143a	0.335838	0.364973	0.31565	0.326891
mmu-mir-200b	0.333706	0.285417	0.255335	0.460367
hsa-mir-30c	0.329496	0.49948	0.274941	0.214066
Near144a1	0.329238	0.095139	0.173315	0.71926
hsa-mir-320	0.329192	0.392449	0.424882	0.170245
miR-300	0.327521	0.385477	0.188597	0.408488
miR-21	0.322805	0.39614	0.229266	0.343009
mmu-mir-126	0.319955	0.268604	0.377971	0.313291
hsa-mir-222	0.318486	0.357182	0.318227	0.280048
mmu-mir-191	0.316664	0.332987	0.245285	0.371719
miR-300	0.31565	0.317404	0.377704	0.251842
mmu-mir-145	0.314111	0.292389	0.199149	0.450797
hsa-mir-26b	0.313623	0.381377	0.083006	0.476485
mmu-mir-145	0.309683	0.294439	0.218062	0.416546
miR-300	0.303646	0.376046	0.218579	0.316313
miR-297	0.30299	0.531877	0.015447	0.361645
CAND707-2	0.302518	0.366204	0.399311	0.142039
hsa-mir-10a	0.301446	0.468314	0.098053	0.337972
rno-miR-351	0.299723	0.363333	0.351991	0.183845
CAND707-2	0.299372	0.316584	0.417835	0.163697
rno-miR-151*	0.297209	0.275985	0.385457	0.230183
mmu-mir-188	0.292837	0.312483	0.229568	0.336461
rno-miR-335	0.291245	0.214883	0.378803	0.280048
hsa-mir-107	0.29124	0.364153	0.253192	0.256375
pred00273	0.288551	0.380967	0.203632	0.281056
miR-141a	0.286477	0.330526	0.211079	0.317824
hsa-mir-200b	0.283551	0.193559	0.261702	0.395392
hsa-mir-210	0.283164	0.258352	0.386142	0.204999
hsa-miR-10a-2	0.281785	0.553201	0.198973	0.093181
rno-miR-151*	0.281577	0.225545	0.354249	0.264938
miR-141a	0.281021	0.351441	0.213085	0.278537
hsa-miR-10a-2	0.280538	0.491279	0.239526	0.11081
miR-34a	0.279431	0.264503	0.327993	0.245798
rno-miR-335	0.278223	0.216934	0.413743	0.203992
mmu-mir-195	0.277319	0.326016	0.211286	0.294655
hsa-miR-301	0.276788	0.261633	0.160749	0.407984
rno-miR-352	0.276077	0.49661	0.139215	0.192407
hsa-mir-10a	0.27367	0.442069	0.087308	0.291633
pred00273	0.268887	0.365794	0.186005	0.254864
hsa-mir-19b-1,2	0.266957	0.166083	0.147221	0.487566
mmu-mir-188	0.266601	0.288288	0.230964	0.280552
pred00380	0.265687	0.241539	0.395351	0.160171
mmu-mir-134	0.264345	0.34857	0.197659	0.246805
miR-34a	0.260733	0.271475	0.29263	0.218095
hsa-mir-213	0.260573	0.295259	0.20893	0.27753
rno-miR-335	0.257451	0.301001	0.278945	0.192407
mmu-mir-30b	0.256277	0.225135	0.364383	0.179311
mmu-mir-126	0.251019	0.295669	0.237279	0.22011
hsa-mir-31	0.247198	0.224315	0.295658	0.221621
hsa-mir-19a	0.246622	0.214063	0.186824	0.338979
hsa-mir-222	0.246614	0.236618	0.254909	0.248316
hsa-miR-301	0.245571	0.246049	0.121463	0.3692
mmu-mir-134	0.244795	0.273935	0.2212	0.23925
mmu-mir-188	0.242888	0.241128	0.169207	0.318328
rno-miR-352	0.241978	0.404751	0.120212	0.20097
rno-miR-140*	0.240307	0.2481	0.288977	0.183845
hsa-miR-342	0.237886	0.230466	0.347198	0.135995
rno-miR-140*	0.233586	0.222675	0.229264	0.24882
hsa-mir-30c	0.233007	0.34898	0.197927	0.152113
miR-34a	0.231505	0.284597	0.233125	0.176793
miR-296	0.230936	0.243179	0.235059	0.214569
miR-34a	0.228519	0.259172	0.261679	0.164705

Table S1. Continued

Name	Avg C	C2	C4	C5
pred00380	0.227591	0.189048	0.33154	0.162186
mir-296	0.227555	0.256712	0.229014	0.19694
hsa-mir-200b	0.227332	0.223495	0.157803	0.300699
hsa-mir-19b-1,2	0.227079	0.144759	0.106332	0.430146
mmu-mir-191	0.226147	0.307562	0.209702	0.161179
hsa-mir-213	0.225167	0.227186	0.1864	0.261916
mmu-mir-125b	0.223641	0.308792	0.216062	0.146068
rno-miR-151*	0.218638	0.24892	0.233726	0.173267
miR-130b	0.217429	0.204631	0.269855	0.1778
mmu-mir-125b	0.215199	0.272295	0.235798	0.137506
hsa-mir-10a	0.214038	0.363743	0.067831	0.21054
rno-miR-335	0.213828	0.227596	0.192772	0.221117
hsa-mir-25	0.212078	0.267784	0.171511	0.19694
hsa-mir-213	0.212031	0.282547	0.197908	0.155638
mmu-mir-125b	0.209624	0.289108	0.228954	0.11081
mmu-mir-194	0.20624	0.255481	0.218177	0.145061
rno-miR-324-5p	0.205904	0.159522	0.281396	0.176793
mmu-mir-125b	0.205319	0.271064	0.187742	0.157149
mmu-mir-194	0.203422	0.220624	0.226953	0.16269
miR-130	0.199704	0.182077	0.231679	0.185356
mmu-mir-194	0.199207	0.24974	0.19174	0.156142
hsa-mir-19a	0.196742	0.175925	0.136771	0.27753
miR-296	0.196452	0.205041	0.207523	0.176793
miR-172	0.194955	0.178386	0.255878	0.150601
mmu-mir-152	0.193471	0.225545	0.083887	0.270982
mmu-mir-188	0.192858	0.221855	0.144668	0.212051
hsa-mir-15	0.19216	0.1993	0.138937	0.238242
rno-miR-322	0.191902	0.14722	0.295513	0.132973
hsa-mir-25	0.191718	0.177566	0.154309	0.243279
hsa-mir-10a	0.190874	0.341599	0.037609	0.193415
miR-296	0.190358	0.207912	0.205508	0.157653
mmu-mir-200b	0.189836	0.196429	0.221975	0.151105
mmu-mir-134	0.188035	0.237848	0.167598	0.15866
mmu-mir-191	0.18469	0.19766	0.157958	0.198451
mmu-mir-194	0.184239	0.212423	0.215883	0.12441
miR-172	0.183046	0.205451	0.192077	0.151609
hsa-mir-92-1,2	0.181567	0.161572	0.27131	0.111818
miR-130	0.179831	0.189458	0.232678	0.117358
hsa-mir-213	0.179631	0.20135	0.199533	0.138009
miR-297	0.178205	0.233747	0.032908	0.26796
hsa-mir-216	0.176665	0.19971	0.251205	0.079078
rno-miR-7	0.175717	0.145579	0.194706	0.186867
miR-142s	0.174154	0.145579	0.234339	0.142543
miR-130b	0.172657	0.176746	0.210772	0.130454
hsa-mir-216	0.171388	0.184127	0.24542	0.084619
rno-miR-151*	0.16744	0.183717	0.16649	0.152113
hsa-miR-382	0.165922	0.270244	0.113186	0.114336
hsa-mir-216	0.165156	0.191919	0.20936	0.094189
hsa-miR-382	0.164933	0.207092	0.168837	0.118869
mmu-mir-30b	0.1642	0.14886	0.188102	0.155638
hsa-mir-19b-1,2	0.15892	0.112773	0.091494	0.272493
hsa-mir-15	0.158891	0.180026	0.091145	0.205503
miR-142s	0.157897	0.110312	0.220332	0.143046
hsa-mir-222	0.157817	0.209552	0.114304	0.149594
rno-miR-301	0.155244	0.133687	0.213177	0.118869
hsa-mir-92-1,2	0.15524	0.14681	0.245875	0.073034
hsa-mir-15	0.153924	0.175515	0.154796	0.131461
rno-miR-324-5p	0.151673	0.123025	0.237303	0.094693
hsa-mir-19a	0.151531	0.130816	0.118272	0.205503
mmu-mir-152	0.151005	0.216113	0.092847	0.144054
hsa-mir-15	0.147291	0.229236	0.097798	0.11484
mmu-mir-191	0.146617	0.175515	0.154028	0.110307
rno-miR-140*	0.145345	0.161162	0.114197	0.160675
hsa-mir-100	0.145104	0.081606	0.261029	0.092678
miR-297	0.144701	0.234977	0.051041	0.148083
hsa-miR-379	0.144511	0.142299	0.210142	0.081093
miR-15a	0.141802	0.123025	0.083278	0.219102
rno-miR-140*	0.141774	0.170594	0.136361	0.118366
miR-130b	0.140971	0.171825	0.139774	0.111314
hsa-mir-25	0.140638	0.192329	0.12381	0.105774
miR-130b	0.13945	0.193969	0.157391	0.06699

Table S1. Continued

Name	Avg C	C2	C4	C5
pred00169	0.138051	0.14886	0.160023	0.10527
hsa-mir-100	0.136646	0.126715	0.160826	0.122395
mmu-mir-99a	0.136249	0.145989	0.098053	0.164705
hsa-mir-100	0.135185	0.1017	0.2142	0.089656
hsa-mir-92-1,2	0.134963	0.14804	0.173743	0.083108
hsa-mir-32	0.133832	0.143939	0.130627	0.126928
hsa-mir-148	0.133369	0.113593	0.185274	0.10124
rno-miR-322	0.13332	0.112363	0.19492	0.092678
hsa-mir-200b	0.131201	0.089808	0.14161	0.162186
mmu-mir-15b	0.129998	0.136147	0.134977	0.118869
rno-miR-7	0.129489	0.132457	0.135127	0.120884
hsa-mir-19b-1,2	0.129329	0.088168	0.060064	0.239753
mmu-mir-10b	0.129043	0.183717	0.091089	0.112321
rno-miR-7	0.128136	0.123025	0.129923	0.131461
hsa-mir-221	0.127829	0.164443	0.079524	0.13952
miR-15a	0.127644	0.119334	0.121559	0.142039
hsa-mir-32	0.126964	0.132867	0.140236	0.107788
mmu-mir-10b	0.126742	0.15132	0.117591	0.111314
mmu-mir-10b	0.126642	0.139018	0.125564	0.115344
hsa-mir-19a	0.12576	0.114003	0.078425	0.184852
hsa-miR-361	0.125123	0.172645	0.092921	0.109803
hsa-mir-32	0.125048	0.144759	0.129144	0.10124
mmu-mir-99b	0.124392	0.120154	0.145736	0.107285
hsa-miR-379	0.124249	0.143119	0.167675	0.061953
mmu-mir-99b	0.123913	0.166904	0.106112	0.098722
hsa-miR-376a	0.122011	0.089808	0.037982	0.238242
rno-miR-324-5p	0.121704	0.10047	0.155343	0.109299
mmu-mir-99a	0.117268	0.132867	0.150438	0.068501
mmu-mir-10b	0.117107	0.174695	0.091503	0.085123
hsa-mir-100	0.11678	0.108672	0.158056	0.083612
hsa-mir-32	0.115783	0.132047	0.113056	0.102248
miR-172	0.114433	0.130406	0.134319	0.078575
hsa-mir-25	0.112518	0.155011	0.091879	0.090663
hsa-mir-148	0.111311	0.078326	0.133715	0.121891
miR-99b	0.111252	0.164853	0.065145	0.103759
miR-99b	0.110678	0.173875	0.054399	0.103759
mmu-mir-152	0.110485	0.106621	0.060128	0.164705
mmu-mir-15b	0.10801	0.078326	0.117265	0.128439
hsa-mir-148	0.107853	0.146339	0.099088	0.078071
rno-miR-342	0.106686	0.126305	0.071861	0.121891
pred00219	0.104245	0.121384	0.100185	0.091167
pred0029	0.104244	0.081196	0.123747	0.107788
pred0029	0.104229	0.088578	0.125892	0.098218
pred00169	0.102351	0.09801	0.130972	0.078071
hsa-miR-361	0.101338	0.125075	0.097342	0.081597
rno-miR-322	0.100998	0.088168	0.119127	0.0957
pred00219	0.09821	0.120154	0.077264	0.097211
rno-miR-301	0.097507	0.09842	0.135674	0.058427
miR-172	0.095851	0.112773	0.09268	0.0821
hsa-mir-28	0.095099	0.113183	0.063319	0.108796
miR-99b	0.093045	0.142299	0.049698	0.087137
pred00379	0.092903	0.088988	0.063802	0.125921
hsa-mir-99	0.092705	0.110722	0.042982	0.12441
hsa-mir-148	0.08971	0.119744	0.044117	0.10527
hsa-mir-222	0.089663	0.110722	0.08473	0.073538
hsa-miR-378	0.088942	0.115233	0.077047	0.074545
mmu-mir-152	0.088663	0.111132	0.069231	0.085626
rno-miR-344	0.088373	0.096779	0.096817	0.071523
rno-miR-342	0.086638	0.102931	0.051713	0.10527
rno-miR-344	0.086357	0.1017	0.089374	0.067997
hsa-miR-378	0.085741	0.123435	0.070829	0.06296
rno-miR-322	0.085733	0.089398	0.092249	0.075553
pred00379	0.085166	0.09801	0.071861	0.085626
hsa-miR-128ash	0.080846	0.088578	0.051713	0.102248
pred00395	0.080481	0.081606	0.087308	0.07253
rno-miR-301	0.079155	0.079146	0.0737	0.084619
pred00395	0.077491	0.077506	0.067831	0.087137
hsa-mir-99	0.076038	0.095139	0.062458	0.070516
mmu-mir-99b	0.075962	0.076275	0.068503	0.083108
hsa-mir-375	0.073574	0.068484	0.072658	0.079582
mmu-mir-99a	0.07278	0.068074	0.065145	0.085123

Table S1. Continued

Name	Avg C	C2	C4	C5
hsa-mir-28	0.071446	0.084477	0.062367	0.067494
hsa-miR-128b	0.054726	0.054541	0.041639	0.067997

The background-corrected median signal for pixels from each probe spot in the Cy3 channel is shown. Note that several different data points were obtained for most probes. Human and rat as well as mouse miRNAs are included in the microarray, but in general have sequences sufficiently similar to the corresponding mouse miRNA to give a positive signal in this assay. Individual and average data for the control samples are shown after subtraction of background values. C denotes control; Avg denotes average.

at 4°C in ice-cold dispase II solution (2.5 µg/ml in 1×PBS, Roche). Epidermal preparations were lysed in 8M Urea with 4% Chaps in 40 mM Tris. Full-thickness skin was disrupted in liquid nitrogen prior to lysis in 8M urea. Cleared lysates were denatured in 1×LDS sample buffer (Invitrogen), and equal amounts of protein were subjected to SDS polyacrylamide gel electrophoresis followed by transfer to Hybond-P PVDF membrane and incubation with antibodies to cleaved Caspase-3, IKK α , and phosphoHistone H3 (all from Cell Signaling Technology), anti-Dicer serum 349 [S7], or anti-Rac1 (Upstate). Antibodies were visualized with the ECL Plus Western Blotting Detection System (Amersham). Equal loading of control and KO samples was confirmed by Ponceau S staining of the blots. Assays were performed in triplicate. For northern analysis, 2–10 µg of total RNA was loaded on 15% TBE-Urea gels, blotted onto Hybond-N+ membranes (Amersham), and probed with γ -³²P-ATP-labeled antisense oligonucleotides. For semiquantitative RT-PCR, epidermal RNA was isolated with the mirVana RNA extraction kit (Ambion). RNA was incubated with DNaseI and diluted to 30 ng/ μ l. One microliter was used for RT-PCR using the mirVana qRT-PCR detection Kit with mature-miRNA-specific primers (Ambion). PCR products were visualized on 20% TBE polyacrylamide or 2.5% agarose gels. Assays were performed in triplicate. For real-time PCR, RNA was reverse transcribed with random primers and reverse transcriptase (Superarray Bioscience, Frederick, Maryland) in a 20 µl reaction. cDNA was diluted 10-fold, and 1 µl was used as template. The CD34 primer pair PPM04743A was purchased from Superarray Bioscience. Beta-actin was used as the reference standard (normalizer). Twenty-five microliter reactions were performed in triplicate with SYBR green and an MJ Opticon II thermocycler (Bio-rad, Hercules, CA) according to the manufacturer's instructions. Data were analyzed with the Opticon III program.

2D Gel Electrophoresis

Protein concentrations of samples were determined by the Bradford Assay (BioRad). Twenty-five milligrams of control skin protein extract was labeled with 200 pmol Cy3; 25 mg of Dicer mutant skin extract was labeled with 200 pmol Cy5; and 12.5 mg control sample plus 12.5 mg mutant skin sample were labeled with 200 pmol Cy2. Proteins were separated by 2D gel electrophoresis with an 18 cm pH 3–10 nonlinear first-dimension strip and a 20 cm × 25 cm, 10% Tris-Glycine second-dimension gel. Gels were imaged with a Typhoon scanner and analyzed with DeCyder software as instructed by Amersham's DIGE protocol. Experiments were performed in triplicate.

Supplemental References

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Table S2. Ratios of Signal Strengths for miRNA Probes Showing Differential Expression on Microarray Analysis between Control and Dkk1-Expressing Newborn Skin

Name	Ratio TG/C	t Test C versus TG	Cy3(C) #2xb	Cy5(TG) #2xb
ath-miR156	-0.0115124	0.04920303	2	0
ath-miR157	0.01117262	0.01891472	2	0
ath-miR157	0.00308596	0.01934643	2	0
ath-miR157d	0.00569139	0.04150474	2	0
ath-miR157d	0.00364203	0.04154434	2	0
ath-miR158	0.03496322	0.01723526	2	0
ath-miR158	0.00157642	0.02945112	2	0
ath-miR159b	0.1544605	0.02372096	2	0
ath-miR160	0.19785905	0.00332378	2	0
ath-miR160	0.21806865	0.02667875	2	0
hsa-miR-200a	0.29556071	0.00700767	3	3
hsa-miR-200a	0.31157626	0.00805175	3	3
hsa-mir-105-1,2	0.16705433	6.20E-05	2	0
hsa-mir-106	0.60747898	0.01015275	3	3
hsa-mir-106	0.59315019	0.03462643	3	3
hsa-mir-106	0.66384535	0.03803733	3	3
hsa-mir-15	0.52955713	0.01437684	3	3
hsa-mir-20	0.61072124	0.02516693	3	3
hsa-mir-200b	0.24695728	0.02500194	3	2
hsa-mir-200b	0.29261663	0.04239238	3	3
hsa-mir-221	0.26917096	0.01424025	2	0
hsa-mir-222	0.31594874	0.00211031	3	3
hsa-mir-222	0.46606215	0.02910352	3	3
hsa-mir-224	0.09032775	0.00096408	2	0
hsa-mir-224	0.0888662	0.00336122	2	0
hsa-mir-224	0.03327959	0.01161147	2	0
hsa-mir-224	0.02782552	0.01408492	2	0
hsa-mir-32	0.53760466	0.00182812	3	3
hsa-mir-32	0.57128093	0.01928647	3	3
hsa-mir-32	0.58595331	0.02889433	3	3
hsa-mir-32	0.56909911	0.03253958	3	3
miR-106b	0.63038442	0.03190607	3	3
miR-106b	0.62732585	0.04632313	3	3
miR-15a	0.41510391	0.01631789	3	2
miR-15a	0.38406364	0.04466049	2	1
miR-172	0.56210774	0.01921015	3	3
miR-172	0.65251021	0.04630511	3	2
miR-93	0.71712653	0.00924578	3	3
miR-93	0.72170815	0.01018111	3	3
miR-93	0.70241567	0.04697762	3	3
mmu-mir-15b	0.64427942	0.04562302	3	3
mmu-mir-182	0.35040226	0.03007689	2	1
mmu-mir-195	0.37548475	0.01967491	3	3
mmu-mir-195	0.43294566	0.03443767	3	3
mmu-mir-200b	0.48078711	0.03067938	3	3
mmu-mir-200b	0.51093807	0.03258397	3	3
mmu-mir-27b	0.5302577	0.00970043	3	3
mmu-mir-27b	0.51172175	0.03825091	3	3
rno-miR-7	0.64673691	0.00037122	3	3
rno-miR-7	0.76271873	0.00628534	3	3
rno-miR-7	0.64201096	0.04317704	3	3

Probe spots were only included in the analysis if they gave signals that were more than 2-fold above background in at least two of the three control samples and gave statistically higher signal in control samples than in Dkk1-expressing samples ($p < 0.05$) when analyzed by a two-tailed Student's t test. The numbers of samples for each group showing more than 2-fold increased signal over background are displayed in the final two columns. C denotes control; TG denotes Dkk1-expressing transgenic. Additional miRNAs not listed here showed statistically significantly increased expression in control samples compared with Dkk1-expressing samples, but gave signals that were less than 2-fold above background.

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